Localization of Polycyclic Hydrocarbon Carcinogens in the Lung following Intratracheal Instillation in Gelatin Solution

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SUMMARY

The deposition and localization of benzo(a)pyrene (BP) suspended in a gelatin-0.9% NaCl solution was studied in hamster lungs by ultraviolet fluorescence microscopy. BP was deposited primarily in the alveolar region of the lung. Although large numbers of BP-filled macrophages were seen in the upper airways by 24 hr after an instillation, little BP appeared to penetrate into the bronchial epithelium. The intratracheal instillation of polycyclic hydrocarbons in a gelatin-0.9% NaCl solution appears to be a useful model when it is desired to deliver the carcinogen dose to the peripheral lung.

INTRODUCTION

Relatively little attention has been specifically paid to the role of carrier particles and solutions in the induction of tumors of different histological types and locations in the respiratory tract. Henry et al. (4) recently reported that intratracheal instillations of BP* suspended in 0.9% NaCl solution and gelatin resulted in a high percentage of malignant tumors, primarily adenocarcinomas, in the peripheral lung. In contrast, other tumor induction experiments using intratracheally administered BP as the carcinogen have resulted primarily in upper airway lesions. Using ferric oxide particles as the carrier for BP, for example, Saffiotti et al. (12) and Little and O'Toole (7) found a high incidence of tracheobronchial lesions, primarily epidermoid carcinomas. Following administration of BP alone in 0.9% NaCl solution without carrier particles, Feron (1) found a high incidence of malignant tracheal tumors. With other polycyclic hydrocarbon carcinogens such as methylcholanthrene, studies using carrier particles have also resulted in upper airway lesions (8, 9, 14, 15). Following instillations of methylcholanthrene suspended in gelatin and 0.9% NaCl solution, however, Schreiber et al. (13) recently found peripheral, primarily bronchioloalveolar squamous cell tumors, rather than upper airway lesions. They considered the difference in tumor location to be possibly due to the effects of genetic differences in the animals used, microbiological status of the species tested [Schreiber et al. (13) used specific pathogen-free animals], and the difficulty in determining the origin of tumors in animals with end-stage disease.

The question arises as to whether these methods of administering polycyclic hydrocarbons result in sufficiently different deposition patterns within the respiratory tract, such as may explain these observed differences in tumor location and morphology. It seems reasonable that the particle sizes involved could play an important role in the distribution of the carcinogen. Ferric oxide particles in the dry state are well dispersed and of a size normally expected to reach the peripheral lung (98% with a diameter less than 0.75 μm). When mixed with 0.9% NaCl solution, however, clumping of particles occurs, and much of the deposition is on the mucous ciliary escalator of the larger airways (5). We have previously shown that this deposition results in a large carcinogen dose to the upper airway epithelial cells as BP is eluted off the ferric oxide carrier particles during clearance (5). It seems likely that most of the carcinogen deposition in Feron's (1) study was also in the larger airways; without the use of a carrier particle or a surface-active agent, aggregation of BP occurred and resulted in very large particles of BP (up to 80 μm) (1).

The method used by Henry et al. (4) results in a uniform colloid suspension of BP, containing small particles of the carcinogen without aggregation. It seems reasonable that the BP particles in the BP-gelatin-0.9% NaCl solution instillation method may be reaching different cell populations as compared to the BP-ferric oxide and the BP-0.9% NaCl solution methods. In order to determine the localization of BP when given suspended in 0.9% NaCl solution and gelatin, we have performed a serial sacrifice experiment after intratracheal instillation. We have used frozen-section techniques to avoid translocation and loss of the carcinogen and have studied localization by UV fluorescence microscopy since BP is autofluorescent (5).

MATERIALS AND METHODS

Male Syrian golden hamsters weighing 100 to 125 g were obtained from Dennen Animal Industries, Inc., Gloucester, Mass. They were given a single intratracheal instillation of a suspension of 1.6% BP in 0.5% gelatin-0.9% NaCl solution. The suspension was prepared by R. Davies (Chemistry Division, IIT Research Institute, Chicago, Ill.) as has been previously described (4); the particle size distribution of the BP particles, as determined microscopically, was 0.3 to 5.0

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2 The abbreviation used is: BP, benzo(a)pyrene.

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eral alveolar area was adjacent to larger airways in a tissue section, BP particles were never seen to extend into the larger airways. There were also areas of the peripheral lung that had a pale yellow fluorescence without distinct particles.

Twenty-four to 48 Hr. Some particles remained in the alveolar region, greatly reduced in number in comparison to times immediately following the instillation. Large numbers of macrophages containing clumped BP particles were present in the alveolar region and in the larger airway lumina (Fig. 1). Particles of BP did not appear to enter the larger airway epithelial cells during clearance of the macrophages containing BP on the mucus ciliary escalator (Fig. 1, b). Areas of pale yellow fluorescence in the peripheral lung were still present.

Five to 7 Days. A few BP particles were left in the alveolar region. Occasional clumps of BP particles in macrophages in the peripheral lung and in the larger airways were found. Areas of the peripheral lung were pale yellow; some of the fluorescence appeared to be extracellular. In tungsten light these areas appeared yellow-brown.

Two to 4 Weeks to 3 Months. Essentially no BP particles were left in the peripheral lung or in macrophages in the peripheral lung or larger airways. The pale yellow areas in the peripheral lung persisted; however, their color was unstable on exposure to UV. During exposure the pale yellow areas turned blue-green and later to red in some but not all areas; in tungsten light, these areas appeared yellow-brown. This yellow-brown pigment was also observed by Henry et al. (4) long after BP-gelatin-0.9% NaCl solution instillations were given. The significance of these pale yellow fluorescent areas in the lung is not clear, but they very likely represent end products of BP metabolism.

DISCUSSION

It is clear that following intratracheal instillation of BP in gelatin-0.9% NaCl solution suspension the BP is deposited and retained primarily in the alveolar region and is taken up by alveolar cells. This is in contrast to the BP-ferric oxide method in which much of the carcinogen is deposited in the larger airways on the mucous ciliary escalator and enters epithelial cells after elution from the ferric oxide particles (Ref. 5, Figs. 4 to 7). With the BP-gelatin-0.9% NaCl solution method, only small amounts of carcinogen appear to reach the upper airway epithelial cells. These arose from occasional aggregates of BP particles, which were seen in the upper airways soon after instillation. The entry of BP particles into the respiratory epithelium adjacent to these clumps could explain the few malignant tumors of central location observed by Henry et al. (4).

The results of tumor induction experiments are consistent with these findings. Following administration of BP attached to ferric oxide particles, malignant tumors developed in the tracheobronchial region (7, 12), whereas with BP-gelatin-0.9% NaCl solution, the highest incidence of malignant tumors was in the peripheral lung (4). Other polynuclear hydrocarbons appear to follow a similar pattern. When given on carrier particles, methylcholanthrene has produced tracheobronchial lesions in several studies (8, 9, 14, 15). When methylcholanthrene was suspended in 0.9% NaCl solution and gelatin, however, peripheral tumors developed (13). The method of administration of a respiratory carcinogen thus appears to play a central role in determining the location and histopathology of the induced tumors.

In the present frozen-section BP localization study, the BP particles were initially seen as intense white-yellow fluorescent particles by UV fluorescence microscopy. The development of pale yellow fluorescent areas in the alveolar region within 2 hr after instillation suggests that some in situ metabolism of BP may have occurred. In our earlier study of localization of BP following administration attached to ferric oxide carrier particles (5), a pale yellow fluorescence was also seen in the epithelium and underlying connective tissue of the larger airways (including the trachea). In addition, however, red-orange and blue-green fluorescent products were seen in the upper airways after BP-ferric oxide administration (unpublished observation); these products were not seen in the alveolar region after BP-gelatin-0.9% NaCl solution administration. This difference in fluorescence of by-products suggests that a difference may exist in the ability of different sites in the lung to metabolize BP. Thus, there may be regional differences in the lung that can affect the activity or potency of BP as a carcinogen.

The method of administering a carcinogen also appears to influence its retention. It is clear that the administration of BP on carrier particles such as ferric oxide results in much
longer retention times for the carcinogen than when BP is given as a gelatin-0.9% NaCl solution suspension (3, 4). Clearance rates for the BP-gelatin-0.9% NaCl solution suspension are very fast; Henry et al. (4) report that 95% of a single dose of 3 mg was cleared within 5 hr of the instillation. Similar results have been reported by Kuschner (6). In contrast, when BP (4 mg) was administered on ferric oxide particles, Henry and Kaufman (3) reported that 95% clearance did not occur until 14 hr or later, depending on the size of particles used. Saffiotti et al. (11) found even longer retention times after single doses of BP on ferric oxide particles. We have now demonstrated that carcinogen deposition and localization differs when BP is given as a gelatin-0.9% NaCl solution suspension rather than on carrier particles. The fact that BP retention is greater when ferric oxide particles are used implies either that there is a slower metabolism of BP in the large airways as compared to the alveolar region or that the blood is less efficient at clearing centrally deposited BP.

The role of particle size and character in relation to the retention of BP has recently been reviewed (3). Henry and Kaufman (3) found no significant differences in retention rates of BP when different-sized particles of ferric oxide (range, 0.5 to 30 μm) were used. This is in contrast to the previously reported results of Saffiotti (10), who found that retention increased with increasing particle size. Our experience with instillation of BP on ferric oxide particles also suggests that particle size may not be important in determining carcinogen deposition. Our ferric oxide method uses small particles (98% with a diameter less than 0.75 μm) that, in the dry state, are of a size that should normally be deposited in the peripheral lung. However, when mixed with 0.9% NaCl solution, they aggregate and behave as much larger particles would. In fact, we have found that the ferric oxide particles are deposited primarily on the epithelium of the larger airways (5). Thus, the deposition pattern is characteristic of large particles even though the particles were small before suspension in 0.9% NaCl solution. Small particles (0.3 to 5.0 μm with 50% of the particles in the 0.5 to 1.0 μm size range) are also utilized in the BP-gelatin-0.9% NaCl solution method (4). It appears that gelatin stabilizes this suspension by maintaining a dispersion of small particles, which we found to result in deposition and retention of the carcinogen in the alveolar region. Thus, an alveolar deposition may not be a unique feature of the BP-gelatin-0.9% NaCl solution system but a result of using a stable, small particle suspension.

Another factor that may influence deposition and retention of BP-gelatin-0.9% NaCl solution is concentration. The concentration we have reported (1.6%) is considerably lower than the 6.6% suspension used by Henry et al. (4). We have also studied localization of a 0.67% BP-gelatin-0.9% NaCl solution suspension, used in connection with ongoing low-dose BP and 210Po synergism experiments. This concentration resulted in the same deposition pattern, but the BP appeared to be cleared at a somewhat faster rate than following administration in a 1.6% solution. This observation is consistent with the retention data of Henry et al. (4); they reported that lower doses of BP-gelatin-0.9% NaCl solution were cleared more quickly than high doses. Whether the more concentrated BP suspensions used by Henry et al. (4) in tumor induction experiments would have a greater likelihood of particle aggregation and subsequent deposition in larger airways is unclear. However, since 6.6 to 7.8% BP suspensions resulted primarily in malignant tumors of the peripheral lung (4), it seems most likely that the major site of deposition and retention of carcinogen was still in the alveolar region.

Whether tumors will develop at all is largely dependent on the carcinogen dose and its retention in a given region. The lack of success of BP-0.9% NaCl solution models in tumor production in earlier studies has been attributed to a low effective dose owing to rapid clearance of the carcinogen (2, 6, 16). Our present study suggests that it is not so much the total administered dose but rather the amount reaching and acting at a given site in the lung that determines whether tumors will develop. Clearly, the major carcinogen dose using the BP-gelatin-0.9% NaCl solution technique is delivered to the alveolar region. This model would thus be useful for studying interactions between BP and other carcinogens that also reach the peripheral lung.

REFERENCES

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Fig. 1. a, tungsten light photomicrograph of large bronchioles showing lumina filled with macrophages from the alveolar region. Dark speckled areas within lumen represent BP particles within macrophages. These areas appear yellow-brown in these sections (24 hr after instillation of BP-gelatin-0.9% NaCl solution). Thionin, × 350. b, UV fluorescent photomicrograph of same field as in a showing intense fluorescence of BP particles in macrophages within lumina. Note lack of fluorescence within bronchiolar epithelium.
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